

AMENDMENTS TO THE SPECIFICATION

Please amend the Specification as indicated in the replacement paragraphs below:

Please delete the paragraphs at page 5, line 27 to page 7, line 13.

Please amend the paragraph at page 8, lines 3-5, to read as follows:

In accordance with an eighth embodiment of the present invention, there is provided a novel levan fructotransferase of SEQ ID NO: 1. ~~the following amino acid sequence 1:~~

Please delete the paragraph of sequence data at page 8, lines 7-24.

Please amend the paragraph at page 8, lines 26-27, to read as follows:

In accordance with a ninth embodiment of the present invention, there is provided a novel levan fructotransferase polynucleotide of SEQ ID NO: 2. ~~gene of sequence 2:~~

Please delete the paragraph of sequence data at page 8, line 29 to page 9, line 18.

Please amend the paragraph at page 9, lines 20-21, to read as follows:

In accordance with a tenth embodiment of the present invention, there is provided a novel levan fructotransferase of SEQ ID NO: 1 encoded by a polynucleotide of SEQ ID NO: 2, as provided in the composite polynucleotide/amino acid sequence of SEQ ID NO: 3. ~~gene of sequence 3.~~

Please delete the paragraph of sequence data at page 9, line 23 to page 10, line 34.

Please delete the paragraph at page 12, lines 10-11.

Please amend the paragraph at page 12, lines 18-19, to read as follows:

Fig. 6 shows a nucleotide base sequence of the levan fructotransferase gene (SEQ ID NO: 2) and an amino acid sequence (SEQ ID NO: 1) deduced therefrom, with translation start and restriction sites indicated.

Please delete the paragraph at page 13, line 2.

Please amend the paragraph at page 25, lines 8-21, to read as follows:

Delete plasmids, which were constructed from the 1.6 kb DNA fragments with the aid of an exo/Mungbean delete kit (Takara, Japan), were amplified. 2 μ l of the prepared plasmids prepared from the mutant cells was denatured with 2 N NaOH, neutralized with ammonium acetate (pH 4.5), allowed to precipitate by ethanol, and dissolved in deionized water. Using a Sequenase kit (USB, U.S.A.), the denatured plasmids were labeled and 4 μ l of the reaction was boiled for 3 min and electrophoresed on 8% polyacrylamide-8M urea gel at 1500-1700 V for 3 hours. The gel was dried and exposed to X-ray film at -70°C for about 8 hours. The reading of the base sequence which appeared on the sensitized X-ray film revealed that the total levansucrase gene including its one termination codon is 1,981 bp long: the structural gene corresponding to the amino acid sequence is 1269 bp long. ~~The base sequence of the levansucrase gene and the amino acid sequence deduced therefrom are as shown in Fig. 3.~~

Please amend the paragraph at page x, lines y, to read as follows:

With the aid of a protein-peptide sequencing system (Applied Biosystems, Model 477A), the amino acid sequence of the purified levansucrase was determined at its N-terminal in the Edman degradation procedure. As a result, a stretch of seven amino acid residues ~~, Met-Leu-Asn-Lys-Ala-Gly-Ile,~~ was sequenced, reflecting

the corresponding base sequence of the DNA. In particular, the levU gene was revealed to have no base sequences which correspond to the signal peptides, which are usually found in secretory proteins. The nucleotide and amino acid sequences of the levansucrase gene from *Z. mobilis* was registered in the GenBank, U.S.A. (Accession No. AF081588).

Please amend the paragraphs at page 27, lines 21-26, to read as follows:

A levansucrase gene 1296 bp long was amplified by a PCR which employed two primers ~~the following primers 1 and 2~~ with the plasmid pZL 8 serving as a template. [[:]]

~~Primer 1: 5'-CGC CGG ATC CAC ATG TTG AAT AAA GCA GGC-3'~~

~~Primer 2: 5'-CGC GGA TCC ACA TGT GCA TAA TCA GAA ACG TC-3'~~

Please amend the paragraph at page 31, lines 1-8, to read as follows:

A PCR was carried out using two primers ~~Primer 1 and Primer 3~~ ~~(5'-CGA CAT GTT AGT GAT GGT GAT GGT GAT GTA AAG ACA GGG CTG-3')~~ while the plasmid pZL8 isolated from *E. coli* KCTC 8546P served as a template. Using the PCR product, the same procedure as Experimental Example 2 was repeated to construct the plasmid pEL12 capable of expressing the levansucrase carrying histidine residues at its C-terminus in *E. coli*, which was then used for the

transformation of *E. coli* BL21 (DE3).

Please amend the paragraph at page 39, lines 2-12, to read as follows:

The purified enzyme was 51,000 in molecular weight as measured by SDS-PAGE while being measured to have a molecular weight of 96,000 by gel filtration chromatography. Thus, it was identified as being a dimer in aqueous conditions. An amino acid analysis showed ~~that the amino acid sequence of the C-terminus of the enzyme~~ had at its C-terminus a stretch of the amino acid sequence ~~SAPGSLRAVYHMTTPPSGXLXDOQ wherein X residues are not identified.~~ Maintaining its stability in a pH range of 4-10.5, the enzyme showed optimal activity at around pH 6.0. Also, it was of optimal activity at 55°C. After being allowed to stand at 50°C for 30 min, the enzyme was observed to have a remnant activity of 90 % or greater (Table 9). Whereas being inhibited by Mn^{2+} , Fe^{2+} and Hg^{2+} , the activity of the enzyme was enhanced by Na^{2+} and Ca^{2+} .

Please amend the paragraph at page 41, line 3 to page 42, line 6, to read as follows:

First, a sequence ~~the V-Y-H-T-P~~ in the N-terminal amino acid sequence of the K2032 strain and a second ~~the~~ amino acid sequence ~~(-E-C-P-D-L-F, p2 primer)~~, which was found to be in a homology relationship with levan fructotransferase, were used to

synthesize degenerated primers (~~p1: 5'-GTNTAYCAYATGACNCC and p2: 5'-AABAYYTCSGGYCARTC~~). Using these primers, a standard PCR was carried out with the genomic DNA of K2032 bacteria serving as a template. The PCR products ranging, in size, from 600 to 650 bp were eluted from the gel and introduced into shuttle vectors for *E. coli*. As a result of restriction enzyme gene mapping, the DNA fragments introduced were identified as being divided into four kinds and the vectors carrying these DNA fragments were called pDA11, 17, 18 and c8, respectively. In addition, base sequencing analysis of the four DNA fragments showed that the DNA fragment inserted in pDA11 and the levan fructotransferase gene of *A. nicotinovorans* are in high homology (85%) relationship. Therefore, the DNA fragment introduced in pDA18 could be used as a probe for the Southern hybridization with the DNA prepared from *A. ureafaciens* K2032 with the aim of detecting the levan fructotransferase gene anchored in the bacteria. Before the Southern hybridization, the genomic DNA of *A. ureafaciens* K2032 was digested with various restriction enzymes. As a result of the Southern hybridization, a signal was detected in a 5.6 kb DNA fragment upon digestion with *ClaI*, in a 8.0 kb DNA fragment upon digestion with *PstI*, and a DNA fragment longer than 10 kb upon digestion with *BamHI*. Accordingly, the genomic DNA was cut with either *ClaI* or *PstI* and electrophoresed on a 1% agarose gel, after

which DNA fragments in a size range of 5-10 kb were eluted from the agarose gel. The partial genomic DNA fragments were ligated into the cloning vector pBluescript KSII⁺ which was previously cut with *ClaI* or *PstI*. These recombinant plasmids were transformed into *E. coli* DH5 α . Of the transformants, the cells carrying a gene coding for a levan fructotransferase were selected by Southern hybridization. Finally, when cutting with *ClaI*, there was obtained plasmid pDCla carrying a 5.6 kb DNA fragment. On the other hand, when cutting with *PstI*, there was obtained plasmid pDpst carrying a 8.0 kb DNA fragment.